REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED *(From - To)***
2009	Open Literature	

4. TITLE AND SUBTITLE
Therapeutic options to treat sulfur mustard poisoning—The road ahead

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Smith, WJ

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDR
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER

USAMRICD-P08-013

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDZ-I
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
Published in Toxicology 263, 70–73, 2009

14. ABSTRACT
See reprint.

15. SUBJECT TERMS
Sulfur mustard, HD, Therapeutics, Treatments, Acute injury, treatments, chemical warfare agent

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			William J. Smith
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	4	**19b. TELEPHONE NUMBER *(include area code)*** 410-436-4255

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Toxicology 263 (2009) 70–73

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Review

Therapeutic options to treat sulfur mustard poisoning—The road ahead

William J. Smith [1]

Research Division, US Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, United States

ARTICLE INFO

Article history:
Received 30 June 2008
Received in revised form 9 September 2008
Accepted 12 September 2008
Available online 21 September 2008

Keywords:
Sulfur mustard
HD
Therapeutics
Treatments
Acute injury

ABSTRACT

For the past 15 years the international research community has conducted a basic and applied research program aimed at identifying a medical countermeasure against chemical threat vesicant, or blistering, agents. The primary emphasis of this program has been the development of therapeutic protection against sulfur mustard and its cutaneous pathology—blister formation. In addition to the work on a medical countermeasures, significant research has been conducted on the development of topical skin protectants and medical strategies for wound healing. This review will focus on the pharmacological strategies investigated, novel therapeutic targets currently under investigation and therapeutic approaches being considered for transition to advanced development. Additionally, we will review the expansion of our understanding of the pathophysiological mechanisms of mustard injury that has come from this research. While great strides have been made through these investigations, the complexity of the mustard insult demands that further studies extend the inroads made and point the way toward better understanding of cellular and tissue disruptions caused by sulfur mustard.

Published by Elsevier Ireland Ltd.

Contents

1. Introduction

Sulfur mustard (Army designation HD; mustard gas) is an alkylating agent with cytotoxic, mutagenic and vesicating properties. Its use on the battlefield results in debilitating injuries to skin, eyes and the respiratory system (Papirmeister et al., 1991; Smith and Dunn, 1991). While many of the toxic manifestations that follow HD exposure to cells and tissues have been defined, the underlying mechanisms of pathology remain elusive. Much of the research in this area has been conducted in the laboratories of the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD), the laboratories of our NATO allies, and academic and industrial laboratories funded through Army and Department of Defense (DoD) extramural contract programs. Based on the technological database developed through this program, we have been able to generate a unifying hypothesis for cellular and tissue events that explains the formation of cutaneous blisters following exposure to HD. Studies of individual toxic events, such as alkylation of cellular macromolecules, formation of DNA strand breaks, activation of poly(ADP-ribose) polymerase (PARP or PADPRP), disruption of calcium regulation, proteolytic activation and tissue inflammation, have together led to the formulation of six strategies for therapeutic intervention (Smith et al., 1996, 1999). The proposed pharmaceutical strategies are intracellular scavengers, DNA cell cycle modulators, PARP inhibitors, calcium modulators, protease inhibitors and anti-inflammatory compounds.

These compound classes have been evaluated as medical countermeasures against HD dermatotoxicity. For *in vivo* screening, we have utilized the mouse ear vesicant model (MEVM) with

E-mail address: william.j.smith3@us.army.mil.

[1] Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

0300-483X/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.tox.2008.09.012

associated histopathological evaluation (Casillas et al., 1997) and cutaneous HD vapor exposure in hairless guinea pigs (Yourick et al., 1991).

2. Basic research

After the introduction of HD onto the battlefield in World War I and through the 1940s, most of the research efforts directed toward HD focused on defining the histopathological sequelae of exposure in humans. Attempts were also made to establish relevant animal model systems. Beginning in the 1950s, research turned more toward the biochemical effects of HD, and empirical studies were conducted with the aim of identifying therapeutic modalities. While the biochemical studies led to significant inroads for our understanding of the toxic mechanisms, the therapeutic approaches were futile. During the 1960s and 1970s, HD research focused mostly on DNA damage and repair, cytotoxic mechanisms and mutagenesis. Around 1990, the U.S. Army decided to focus its efforts in developing medical intervention strategies for HD injury through the formulation of an Army Science and Technology Objective (STO) titled *Medical Countermeasures Against Vesicant Agents*. A STO is a focused research effort under Army funding that is directed toward attainment of specific target milestones for development of medical products. This STO required successful completion of three technical milestones: (1) define technological and pathophysiological databases and establish pharmacological intervention strategies for the HD injury; (2) show efficacy of a candidate medical countermeasure in an animal model; (3) prepare a Milestone 0 (terminology recently changed to Milestone A) drug development decision (the official decision to move a product from the technical base to the drug development process).

The first technical milestone was met through the research efforts of the USAMRICD, the extramural contract program of the U.S. Army Medical Research and Materiel Command, and the medical research programs of our allied nations. From this research, we were able to construct a schema of the major events of the pathological processes documented in cells and tissues exposed to HD (Fig. 1). This schema was presented at numerous Department of Defense and professional scientific forums, including the 20th Army Science Conference (Smith et al., 1996). The research findings of this program served as part of a NATO-sponsored monograph on HD research (Smith and Mol, 1997).

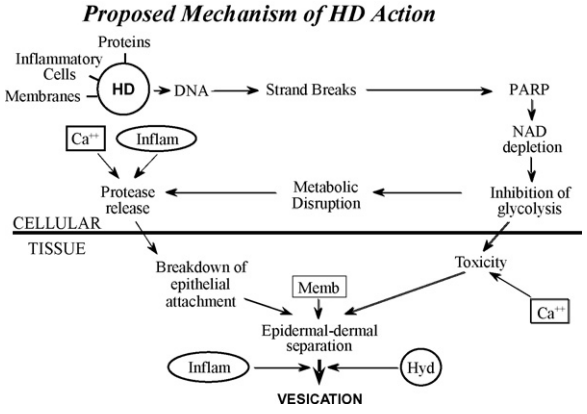

Fig. 1. The cellular and tissue alterations induced by HD that are proposed to result in blister formation. HD can have many direct effects such as alkylation of proteins and membrane components (Memb) as well as activation of inflammatory cells. One of the main macromolecular targets is DNA with subsequent activation of poly(ADP-ribose) polymerase (PARP). Activation of PARP can initiate a series of metabolic changes culminating in protease activation. Within the tissue, the penultimate event is the epidermal–dermal separation that occurs in the lamina lucida of the basement membrane zone. Accompanied by a major inflammatory response (Inflam) and changes in the tissue hydrodynamics (Hyd), fluid fills the cavity formed at this cleavage plane and presents as a blister.

Table 1
Strategies for pharmacologic intervention of the HD lesion.

Biochemical event	Pharmacologic strategy	Example
DNA alkylation	Intracellular scavengers	N-acetyl cysteine
DNA strand breaks	Cell cycle inhibitors	Mimosine
PARP activation	PARP inhibitors	Niacinamide
Disruption of calcium	Calcium modulators	BAPTA[a]
Proteolytic activation	Protease inhibitors	AEBSF[a]
Inflammation	Anti-inflammatories	Indomethacin; Capsaicin Hydrocortisone

[a] BAPTA is a calcium chelator; AEBSF is a sulfonyl fluoride compound.

The latter part of this milestone, i.e., establish pharmacological intervention strategies for the HD injury, was met by utilizing the information developed for the mechanistic schema. We identified six specific areas of the pathologic mechanism that could serve as points of pharmacological intervention into the HD injury. These areas were presented along with the mechanistic schema at numerous meetings and are presented in Table 1 along with prototypic compounds that have been shown to be efficacious against HD toxicity in various model systems.

The second technical milestone called for the demonstration of efficacy by a candidate countermeasure in an animal model. This was first met by research in hairless guinea pigs (Yourick et al., 1991) and subsequently confirmed in the MEVM (Casillas et al., 1997; Smith et al., 1998).

3. Candidate compound screening

In FY97, the U.S. Medical Chemical Defense Research Program was converted from Army funding to Department of Defense funding. The development of a medical countermeasure against vesicant agents was placed under a Defense Technology Objective (DTO) [similar to a STO but signifying control by DoD rather than Army], and while the technical milestones remained intact, a new metric was imposed on the drug development effort. Rather than identifying compounds that just significantly reduced our pathological endpoints, we were required to attain at least a 50% reduction of the indicators of morbidity.

Over 700 candidate prophylactic or therapeutic compounds have been evaluated through the DTO. Sixty-two compounds demonstrated an ability to provide significant modulation of edema and/or histopathology caused by HD *in vivo*. Of these 62 compounds, 19 demonstrated at least 50% reduction of the pathological indicators of mustard injury (Table 2). All of these 19 successful candidates fall into four of our six original proposed strategies: anti-inflammatories ($n = 9$), chemical scavengers ($n = 5$), antiproteases ($n = 3$), or PARP inhibitors ($n = 2$).

The third milestone of the Technical Objective, pass a Milestone 0 drug development decision, was met when we received approval for transition to Concept Development in November 2000. A new DTO was approved with that transition, and research under that DTO drove the drug development process through Concept Exploration toward a transition to Advanced Development. A large effort under the DTO allowed the final selection of two pharmacological approaches to the vesicant injury, i.e., anti-inflammatories and chemical scavengers, which were put forward to our drug development partners. Within each approach, we provided at least two

Table 2
Candidate countermeasures with greater than 50% efficacy in mouse ear model (total significant compounds = 19).

	ICD #	% decrease in histopathology
Anti-inflammatory drug		
Fluphenazine dihydrochloride	2040	50
Indomethacin	2086	96
Olvanil	2723	91
Hydrocortisone	2842	71
Dexamethasone	2845	72
Olvanil (saturated)	2974	53
Retro olvanil	2976	84
Olvanil (urea analog)	2977	81
Octyl homovanillamide	2980	100
Scavenger drugs		
2-Mercaptopyridine-1-oxide	1304	66
6-Methyl-2-mercaptopyridine-1-oxide	1307	56
4-Methyl-2-mercaptopyridine-1-oxide	1308	94
Hydrogen peroxide gel, 3%	2828	58
Dimercaprol	2525	78
Protease inhibitor		
1-(40-Aminophenyl)-3-(4-chlorophenyl) urea	1883	54
N-(0-P)-L-Ala-L-Ala-benzy ester hydrate	2780	62
1(G-T)-4-(4-methyl phenylsemithiocarbazid	2812	50
PARP inhibitor		
3-(4′-Bromophenyl)ureidobenzamide	1548	74
Benzoylene urea	1796	54

candidate compounds for further down-selection based on drug development criteria.

4. Other countermeasure compounds of interest

A number of laboratories not specifically linked to the DTO program have provided research data pointing to therapeutic approaches to the vesicant injury produced by HD. Dr. Uri Wormser and colleagues at the Hebrew University of Jerusalem have published extensively on the efficacy of iodine formulations in protecting against the HD-induced cutaneous injury. While it was initially assumed that the protective effect of iodine was related to its ability to oxidize HD, this effect could not be shown. Wormser identified unusual peptides in the blood of iodine-treated HD-exposed guinea pigs that he suggests are involved in the protective mechanism. A recent paper from his laboratory by Brodsky et al. (2008) summarizes most of the early work and describes the latest studies with the peptides.

For ocular exposures by HD, Babin et al. (2004) from the USAMRICD demonstrated protection, in a rabbit model, against the early stages of HD-induced ocular injury using a sub-Tenons injection of steroid and antibiotic. Investigators from the Israel Institute for Biological Research have evaluated therapeutic candidates in both cutaneous and ocular models of HD exposure. They have seen protection against the cutaneous injury with a combination of anti-inflammatories, the steroid dexamethasone and the non-steroidal diclofenac (Dachir et al., 2002). In their ocular injury model, efficacy was seen using an ophthalmic preparation of anti-inflammatories (Amir et al., 2000).

Lastly, in studies aimed at determining the applicability of therapeutic intervention to molecular signaling pathways disrupted by HD, Dr. Dean Rosenthal of Georgetown University in a collaboration with our institute demonstrated that interfering with Fas receptor-mediated death pathways could totally eliminate the microvesiculation produced by cutaneous HD exposure in both mouse skin and human skin explants on nude mice (Rosenthal et al., 2003).

5. Future

Currently, the selected pharmacological approaches developed under the DTO are under evaluation by the drug developers and funding questions are being resolved. The dilemma faced by the medical chemical defense community is whether the need for protection against vesicant agent injury is still relevant. There is strong support in the military planning community for a decision that vesicants are no longer a concern for our Armed Forces. This is based on highly successful efforts in force protection, i.e., quality chemical protective equipment, sensors, topical skin protection, and military battlefield intelligence. Many in the research community, however, recognize that, while we have done a tremendous job in advancing our understanding of the pathophysiology of mustard, we have much that needs to be done. The following list identifies just a few of the unresolved scientific questions:

- Link cytotoxic mechanisms induced by mustard to production of tissue injury.
- Define novel mechanisms of cell or tissue injury induced by mustard.
- Critically define the chronological events active in cell and tissue injury following exposure to sulfur mustard (HD).
- Associate the histopathological observations in HD-exposed cells and tissues to specific biochemical alterations.
- Establish dose and time relationships for candidate compounds showing efficacy against cell and/or tissue injury induced by HD.
- Identify and study candidate compounds currently undergoing clinical or pre-clinical evaluations in human conditions that may have mechanistic relevancy to HD injury.
- Develop innovative proposals that will potentially accelerate progress toward the strategic plan objectives.

6. Conclusion

For the first time since the introduction of HD onto the battlefield more than 80 years ago, we have the true potential to protect our warfighters against this insidious chemical weapon through pharmacological therapy. Research cannot stop until we completely eradicate the threat of this agent from the military and civilian worlds.

Conflict of interest statement

None.

References

Amir, A., Turetz, J., Chapman, S., Fishbeine, E., Meshulam, J., Sahar, R., Liani, H., Gilat, E., Frishman, G., Kadar, T., 2000. Beneficial effects of topical anti-inflammatory drugs against sulfur mustard-induced ocular lesions in rabbits. J. Appl. Toxicol. 20, S109–S114.

Babin, M.C., Ricketts, K.M., Gazaway, M.Y., Lee, R.B., Sweeney, R.E., Brozetti, J.J., 2004. A combination drug treatment against ocular sulfur mustard injury. J. Toxicol.: Cutan. Ocular Toxicol. 23, 65–75.

Brodsky, B., Erlanger-Rosengarten, A., Proscura, E., Shapira, E., Wormser, U., 2008. From topical antidote against skin irritants to a novel counter-irritating and anti-inflammatory peptide. Toxicol. Appl. Pharmacol. 229, 342–350.

Casillas, R.P., Mitcheltree, L.W., Stemler, F.W., 1997. The mouse ear model of cutaneous sulfur mustard injury. Toxicol. Methods 7, 381–397.

Dachir, S., Fishbeine, E., Meshulam, Y., Sahar, R., Amir, A., Kadar, T., 2002. Potential anti-inflammatory treatments against cutaneous sulfur mustard injury using the mouse ear vesicant model. Hum. Exp. Toxicol. 21, 197–203.

Papirmeister, B., Feister, A.J., Robinson, S.I., Ford, R.D., 1991. Medical Defense Against Mustard Gas: Toxic Mechanisms and Pharmacological Implications. CRC Press, Boca Raton, FL.

Rosenthal, D.S., Velena, A., Chou, F.P., Schlegel, R., Ray, R., Benton, B., Anderson, D., Smith, W.J., Simbulan-Rosenthal, C.M., 2003. Expression of dominant-negative FADD blocks human keratinocyte apoptosis and vesication induced by sulfur mustard. J. Biol. Chem. 278, 8531–8540.

Smith, W.J., Casillas, R.P., Gross, C.L., Koplovitz, I., 1998. Therapeutic approaches to dermatotoxicity by sulfur mustard. Number 122 in Proceedings of US Army MRMC Bioscience Review, Baltimore, MD, May–June. DTIC AD M001167.

Smith, W.J., Dunn, M.A., 1991. Medical defense against blistering chemical warfare agents. Arch. Dermatol. 127, 1207–1213.

Smith, W.J., Martens, M.E., Gross, C.L., Clark, O.E., Cowan, F.C., 1996. Therapeutic approaches to cutaneous injury by sulfur mustard. In: Proceedings of the 20th Army Science Conference, vol. 2, pp. 699–703.

Smith, W.J., Martens, M.E., Gross, C.L., Clark, O.E., Cowan, F.C., Yourick, J.J., 1999. The use of in vitro systems to define therapeutic approaches to cutaneous injury by sulfur mustard. In: Salem, H., Katz, S.A. (Eds.), Toxicity Assessment Alternatives: Methods, Issues, Opportunities. Humana Press, Totowa, NJ, pp. 205–212.

Smith, W.J., Mol, M.A.E., 1997. Progress and future direction of research into the toxicity and treatment of sulfur mustard exposure. NATO Technical Report AC/243 (Panel 8) TR/19.

Yourick, J.J., Clark, C.R., Mitcheltree, L., 1991. Niacinamide pretreatment reduces microvesicle formation in hairless guinea pigs cutaneously exposed to sulfur mustard. Fundam. Appl. Toxicol. 17, 533–542.